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THE TOXICITY OF *GONYAULAX MONILATA* HOWELL TO *MUGIL CEPHALUS*

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ABSTRACT

Laboratory experiments were conducted to determine the effects of *in vitro* cultures of the marine dinoflagellate, *Gonyaulax monilata* Howell, on fishes. Results indicate that the organism produced a substance(s) toxic to fishes and that the toxicity of the medium is increased if the dinoflagellate cytolyses.

INTRODUCTION

Connell and Cross (1950) reported the concurrent appearance of red, luminescent water, large numbers of a marine dinoflagellate, *Gonyaulax* sp., and the mass mortality of fishes in Offats Bayou near Galveston, Texas. They did not identify the species of *Gonyaulax*, but their report contains photographs and drawings of the associated, chain-forming organism. Howell (1953) reported the occurrence of red water, fish mortality and blooms of *Gonyaulax*, which he described as *Gonyaulax monilata*, in a location near Melborne, Florida. Gunter (1942) had previously reported yearly summer mortality of fishes in Offats Bayou during the period from 1936 to 1941 and, according to Connell and Cross, local residents had observed this red water and dead fishes almost every year during the 15–20 years prior to 1949.

During September 1955, we observed "red water" and dead fishes in Offats Bayou. The organism responsible for the water discoloration was *Gonyaulax monilata*. The samples we collected contained between 500 and 1000 organisms per ml. We were able to establish bacterized cultures of this species in the laboratory at that time. Subsequently, mass cultures of *G. monilata* were established and tests were conducted to determine the effects, if any, of the cultures on fishes.

The results of these tests indicate that bacterized cultures of *Gonyaulax monilata* are toxic to fishes and blooms of this organism may be the direct cause of associated fish mortality. The results of these tests were similar, in that the *Gonyaulax* culture was toxic to fishes and the uninoculated media were not. The experiments differed in the size, number and species of fish tested and the time required for the death of fishes to occur. The data from one experiment are presented in this paper.

METHODS AND MATERIALS

The materials used in this experiment consisted of 10 liters of artificial sea water medium (Table 1), 5 liters of which were placed in each of two Pyrex bottles (2½ gal). Both bottles were autoclaved at 15 lbs for 30 min. One of the bottles of medium was inoculated with 10.0 ml of a bacterized culture of *Gonyaulax monilata*. Both the inoculated and uninoculated bottles were placed adjacent to two 40 w, standard, cool white, fluorescent tubes.

After 3 months, at which time the test was conducted, the inoculated medium contained approximately 1,400 *G. monilata* per ml.

Aliquots of both the *G. monilata* culture and uninoculated medium were treated as follows: A) unaltered; B) heated to 50°C and allowed to return to room temperature; C) frozen for 24 hours, thawed, and allowed to return to room temperature; D) aerated throughout the experiment; E) millipore filtered with AA membrane (filtrate); F) millipore filtered culture (residue from E reconstituted in 600 ml of uninoculated medium). The residue of the filtered uninoculated medium was not used.

Young mullet (*Mugil cephalus*) were maintained in aerated sea water for four days prior to the test. For the test, we used 600 ml of each of the 11 test media in one

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TABLE 1. *Composition of artificial sea water medium for growth of bacterized cultures of* Gonyaulax monilata *Howell*

	Amount per Liter[1]
NaCl	24.0 g
KCl	0.6 g
$MgCl_2 . 6H_2O$	4.5 g
$MgSO_4 . 7H_2O$	6.0 g
$CaCl_2$	0.7 g
K_2HPO_4	10.0 mg
KNO_3	10.0 mg
Vitamin B_{12}	1.0 μg
Thiamine HCl	10.0 mg
Biotin	0.5 μg
Sulfides[2]	1.0 ml
Vitamin Mix 8[3]	0.1 ml
Metals T[4]	5.0 ml
Adenine Sulfate	1.0 mg
Tris (Hydroxymethyl) Aminomethane	0.1 g (pH 8.2)
(Ethylenedinitrilo) tetraacetic Acid Disodium Salt	10.0 mg

[1] Raise to one liter with distilled water.

[2] Sulfides: NH_4Cl 0.2 g., KH_2PO_4 0.1 g., $MgCl_2 . 6H_2O$ 0.04 g., $NaHCO_3$ 0.2 g., $Na_2S . 9H_2O$ 0.15 g. Raise to one liter with distilled water.

[3] Vitamin Mix 8: thiamine HCl 20 mg., biotin 50 μg., Vitamin B_{12} 5μg., folic acid 0.25 mg., para-aminobenzoic acid 1.0 mg., nicotinic acid 10 mg., thymine 80 mg., choline 50 mg., inositol 100 mg., putrescine 0.8 mg., riboflavin 0.5 mg., pyridoxine 4.0 mg., pyridoxamine 2.0 mg., orotic acid 26 mg. Raise to 100 ml. with distilled water. (From Provasoli, *et al.*, 1956.)

[4] Metals T

	mg. per 100 ml.
Fe (as $Fe_2(C_4H_4O_6)3$)	5.0
B (as H_3BO_3)	5.0
Se (as H_2SeO_3)	1.0
V (as NH_4VO_3)	0.5
Cr (as K_2CrO_4)	0.2
Mn (as $MnCl_2 . 6H_2O$)	1.0
Ti (as TiO_2)	5.0
Si (as $Na_2SiO_3 . 9H_20$)	5.0
Zr (as $ZrOCl_2 . 8H_2O$)	2.0
Ba (as $BaCl_2$)	1.0

Raise to 100 ml. with distilled water.

liter beakers. Each beaker received six fish (average standard length 19 mm.) and was covered with polyethlyene sheeting. These were placed adjacent to 40 w, standard, cool white, fluorescent tubes. The temperature, at the test site, varied from 70° to 74°F.

Samples from each container were taken for pH determinations before adding the fish and at 7 hours after the start of the experiment. Samples were taken from each container for salinity determination, at termination (24 hours after start) of the experiment and from the stock culture of *G. monilata* and uninoculated medium.

Toxicity was determined by the length of time required for the six *M. cephalus* to die. Each fish was removed from the test material immediately upon death, so that the medium would not become excessively fouled.

RESULTS

A summary of the results of this test is contained in Table 2. As shown in this table, the fish died in less than 4½ hours in all aliquots of the *G. monilata* culture, except the millipore filter residue reconstituted in uninoculated medium. None of the fish died in the uninoculated medium, regardless of its treatment, within the 24-hour test period.

G. monilata culture heated to 50°C appeared to be the most toxic. The average survival time was 45 min. The next most toxic test material was the frozen culture, in which the average survival time of the fish was 56 min. In the millipore filtrate aliquot of the culture, the average survival time was 2 hours and 19 min, and in the unaltered and aerated aliquots of the culture, it was 3 hours and 5 min and 3 hours and 19 min, respectively.

DISCUSSION

Results of this experiment, and the others we conducted, indicate that *Gonyaulax monilata* produces a substance(s) toxic to fishes. Disrupting the dinoflagellates by heating or freezing the cultures appeared to increase this toxicity. The mullet in both heated and frozen *G. monilata* cultures died within one hour, whereas in the untreated culture the first fish did not die until over two hours had elapsed. This would indicate that toxicity does not depend on the presence of living organisms. Similar results with *Gymnodinium breve* were found by Ray and Wilson (1957) and by Starr (1958) who reports that the toxicity of a living culture of *G. breve* is increased by physical and chemical procedures which effectively destroy the organism.

The results indicate that the toxicity of a millipore filtrate is greater than that of the untreated culture, but the residue reconstituted in uninoculated medium did not

TABLE 2. *Effects of bacterized culture of* Gonyaulax monilata *on* Mugil cephalus

Container number and medium		Distress[1] time	Death[2] time	pH[3] before	pH[4] after	Salinity[5]
AI	Unaltered *G. monilata* culture	1:55 2:01 2:49 3:03 3:08 3:09	2:04 2:47 3:02 3:16 3:17 4:03	8.0	7.7	29.62
AII	Unaltered Uninoculated medium	None[6]	None	7.6	6.7	29.46
BI	*G. monilata* culture heated to 50° C., then returned to room temp.	0:31 0:36 0:41 0:41 0:41 0:42	0:36 0:41 0:43 0:45 0:48 0:58	7.9	7.7	27.73
BII	Uninoculated medium heated to 50° C., then returned to room temp.	None	None	7.5	6.6	29.59
CI	*G. monilata* culture frozen for 24 hours, thawed, then returned to room temp.	0:47 0:47 0:48 0:50 0:51 0:51	0:50 0:51 0:57 0:57 0:59 1:00	8.0	7.8	29.51
CII	Uninoculated medium frozen for 24 hours, thawed, then returned to room temp.	None	None	7.5	6.6	29.53
DI	Unaltered *G. monilata* culture aerated during experiment	2:15 2:15 2:51 2:57 3:12 3:22	2:34 2:34 2:58 3:20 4:05 4:25	8.0	7.6	29.64
DII	Unaltered Uninoculated medium aerated during experiment	None	None	7.6	6.8	29.48
EI	Millipore filtrate of *G. monilata* culture (with AA membrane)	1:22 1:34 1:51 2:09 2:44 3:04	1:27 1:42 1:55 2:20 3:09 3:24	7.8	7.8	29.69
EII	Millipore filtrate of uninoculated medium (with AA membrane)	None	None	7.5	6.6	29.45
FI	Residue of Millipore filtered *G. monilata* culture, in uninoculated medium	None	None	7.5	6.6	29.83

[1] Time (hr:min) required for fish to show first visible signs of imbalance.
[2] Time (hr:min) of cessation of opercular movement.
[3] pH taken before fish were added to test medium.
[4] pH taken seven hours after start of experiment.
[5] Salinity samples taken at termination of experiment (24 hrs. after start). Salinity of stock bottle of *G. monilata* culture—32.70. Salinity of stock bottle of Uninoculated medium—32.60.
[6] The notation "None" means that no distress or death occurred within 24 hours after the start of the test.

kill the fish. It is assumed that the organisms were broken up by the suction of millipore filtration allowing more of the toxic portion to pass through the membrane.

Connell and Cross (1950) found that the fishes in Offats Bayou usually died when the *Gonyaulax* bloom "submerged" and suggested that the decrease of oxygen, that accompanied their submergence, was a contributing factor to the fish mortality. Our results indicate that if the submergence was actually the decomposition of *Gonyaulax* cells, fish would die at that time because more toxin(s) would be released. The results of subjecting fish to aerated *G. monilata* cultures indicate that the lack of oxygen was not the cause of fish deaths in this experiment. Maximum death time of fish in the aerated culture was only 22 min longer than in the untreated culture. Ray and Wilson (1957) have eliminated oxygen deficiency as a possible reason for toxicity caused by *Gymnodinium breve*.

Shilo and Aschner (1953), working with *Prymnesium parvum* found that the toxicity of this organism was decreased by mild acidity, oxidizing agents, absorbents, and bacterial growth. McLaughlin (1956) likewise found that cultures of *P. parvum* grown in alkaline media were far more toxic

than if the pH was reduced, but subsequent raising of the pH restored toxicity. Further work is needed with *Gonyaulax monilata* concerning these factors that may influence toxicity.

Symptoms of human shellfish poisoning associated with blooms of *Gonyaulax catenella* and *Gonyaulax tamarensis* are primarily peripheral paralysis and death by respiratory failure (McFarren, *et al.* 1956). *Mugil cephalus* when exposed to *G. monilata* culture, first exhibits distress by frenzied activity, followed by the loss of equilibrium. The fish then turns upside down or on its side and shows slow opercular movements. It may remain in this position, either floating or lying on the bottom of the container, for some time. Immediately before death, there is a violent burst of activity and the fish dies with mouth and opercula open. Since *G. monilata* seems to have a paralyzing effect on mullet, it would be interesting to compare the effects of the toxin(s) produced by *G. catenella* and *G. tamarensis* with *G. monilata* toxin, on mice or other test animals.

Additional tests with bacteria-free cultures are needed to determine the contribution of bacteria to the toxicity of the cultures.

REFERENCES

Connell, C. H., and J. B. Cross. 1950. Mass mortality of fish associated with the protozoan *Gonyaulax* in the Gulf of Mexico. Science, **112:** 359–363.

Gunter, G. 1942. Recurrent summer fish mortalities on the Texas coast. Amer. Midl. Nat., **28:** 631.

Howell, J. F. 1953. *Gonyaulax monilata,* sp. nov., the causative dinoflagellate of a red tide on the east coast of Florida in August-September, 1951. Trans. Amer. Micros. Soc., **72:** 153–156.

McFarren, E. F., M. L. Schafer, J. E. Campbell, K. H. Lewis, R. A. Taft, E. T. Jensen, and E. J. Schantz. 1956. Public Health Significance of Shellfish Poison: A Review of Literature and Unpublished Research. Proc. Nat. Shellfish Ass., **47:** 114–141.

McLaughlin, J. J. A. 1956. The physiology and nutritional requirements of some chrysomonads. Ph. D. Thesis, New York Univ., 65 pp.

Provasoli, L., J. J. A. McLaughlin, and M. R. Droop. 1957. The development of artificial media for marine algae. Arch. Mikrobiol., **25:** 392–428.

Ray, S. M., and W. B. Wilson. 1957. Effects of unialgal and bacteria-free cultures of *Gymnodinium breve* on fish. U. S. Fish & Widl. Serv., Fish. Bull., **123**, 57: 469–495.

Shilo (Shelubsky), M., and M. Aschner. 1953. Factors governing the toxicity of cultures containing the phytoflagellate *Prymnesium parvum* Carter. J. Gen. Microbiol., **8:** 333–343.

Starr, T. J. 1958. Notes on a toxin from *Gymnodinium breve.* Tex. Rep. Biol. Med., **16:** 500–507.